Delta-Tocotrienol: Radiation Protection and Effects on Signal Transduction Pathways

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Srinivasan (part 1)

Prophylactic and mitigatory studies in CD2F1 mice

Xiao (part 2)

***In vivo* and *in vitro* hematopoiesis and effects of DT3 on Erk/mTOR signaling pathway regulation**

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June 15, 2011

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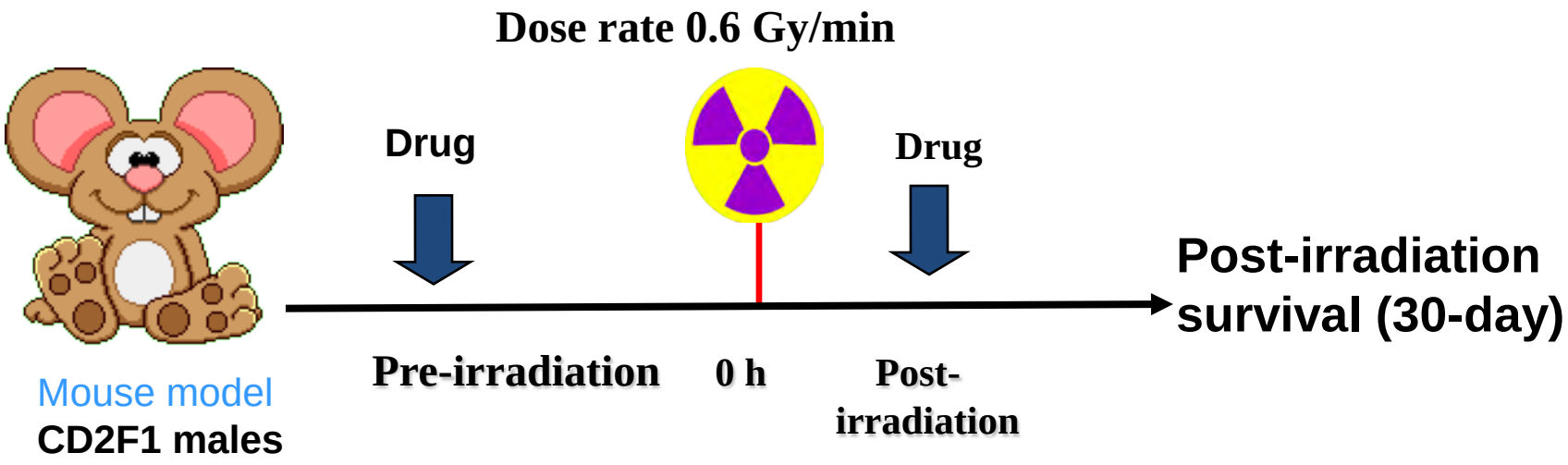
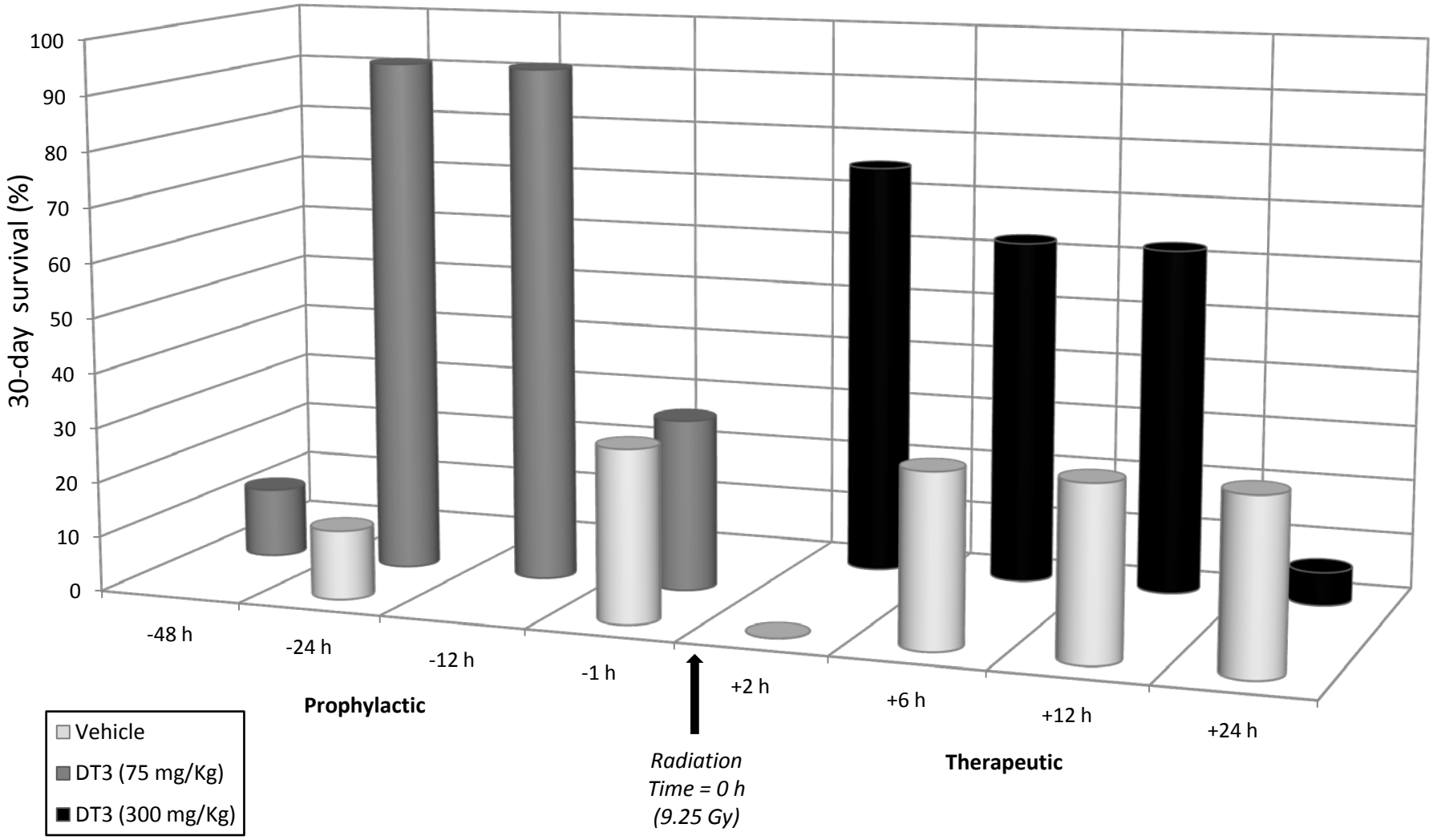
Experimental Design (Radiation survival studies)

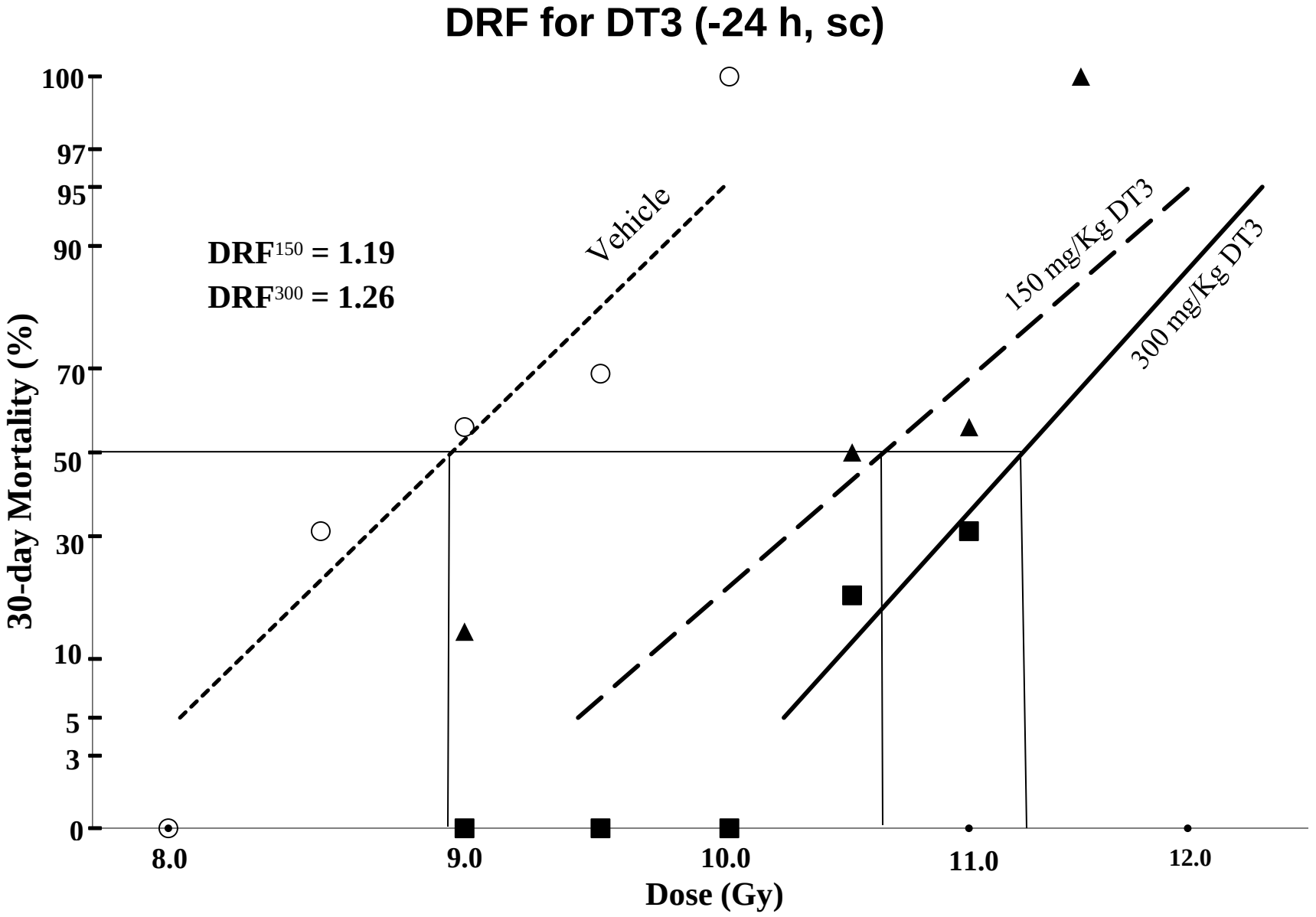


1. **Positive control=5-AED and vehicle PEG-400; 24 h pre-TBI**
2. **12-14 week old**
3. **Cobalt 60 gamma radiation**

Time Optimization with DT3



Satyamitra et al 2011 *R ad Res 175, 736*



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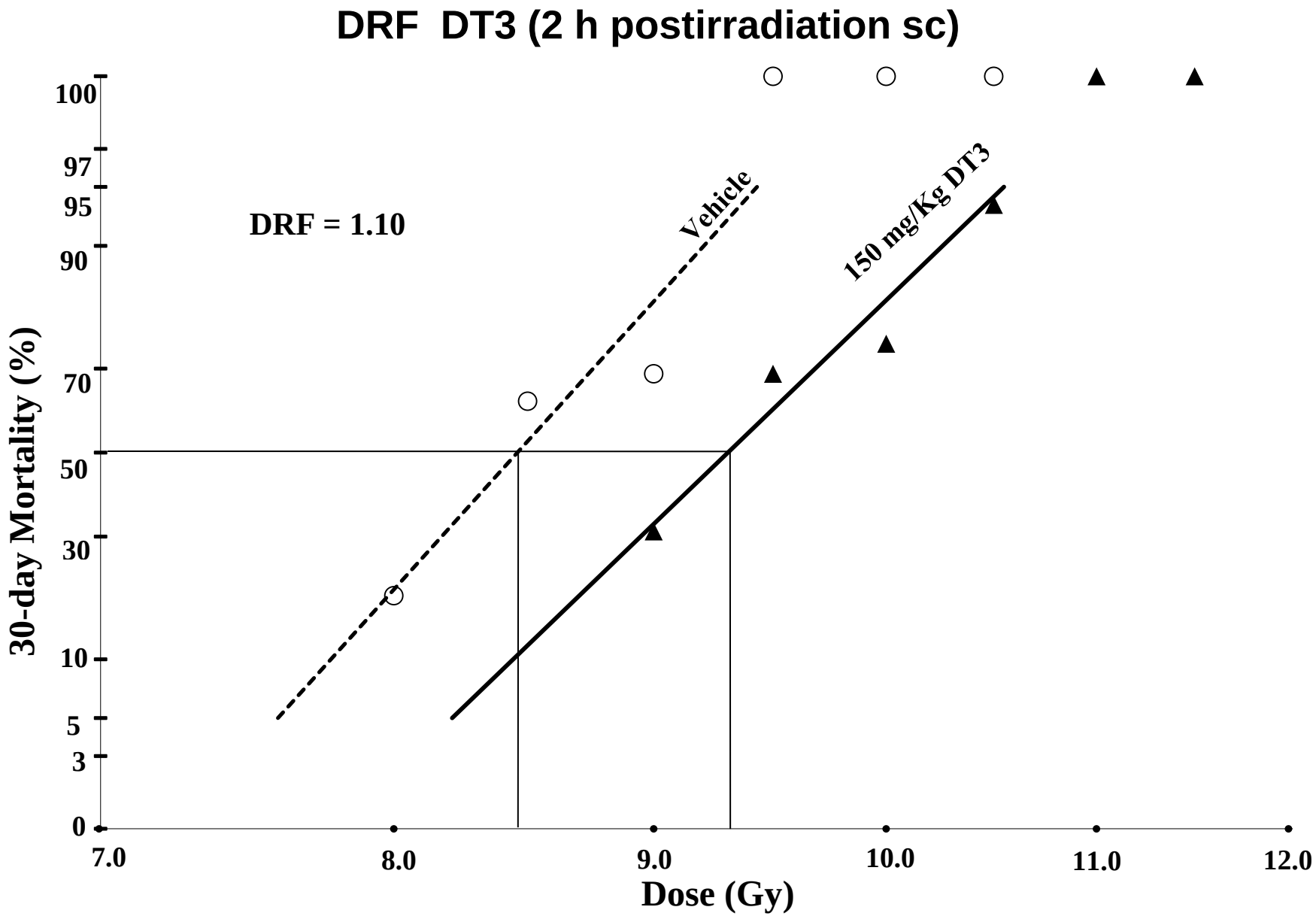

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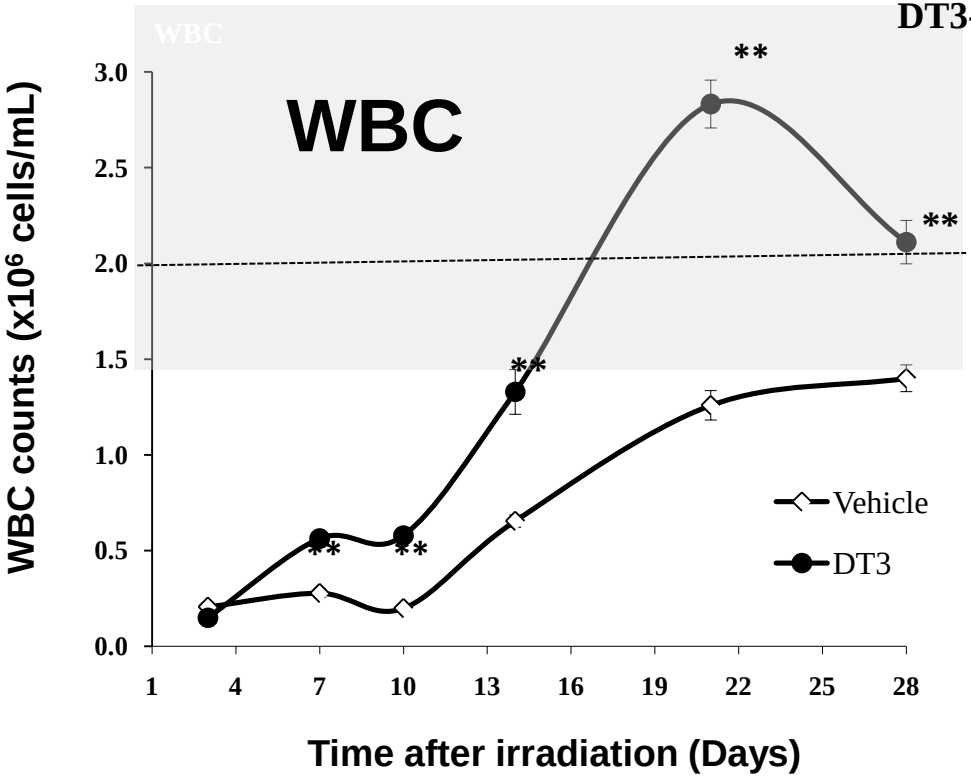
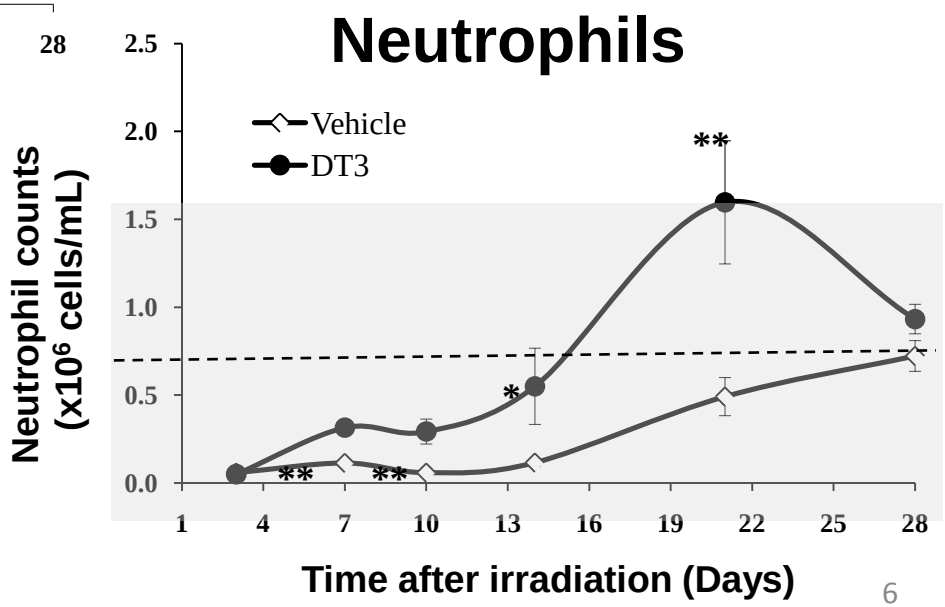
Hematology

DT3- 300 mg/kg, -24 h, sc , 7 Gy @ 0.6 Gy/min





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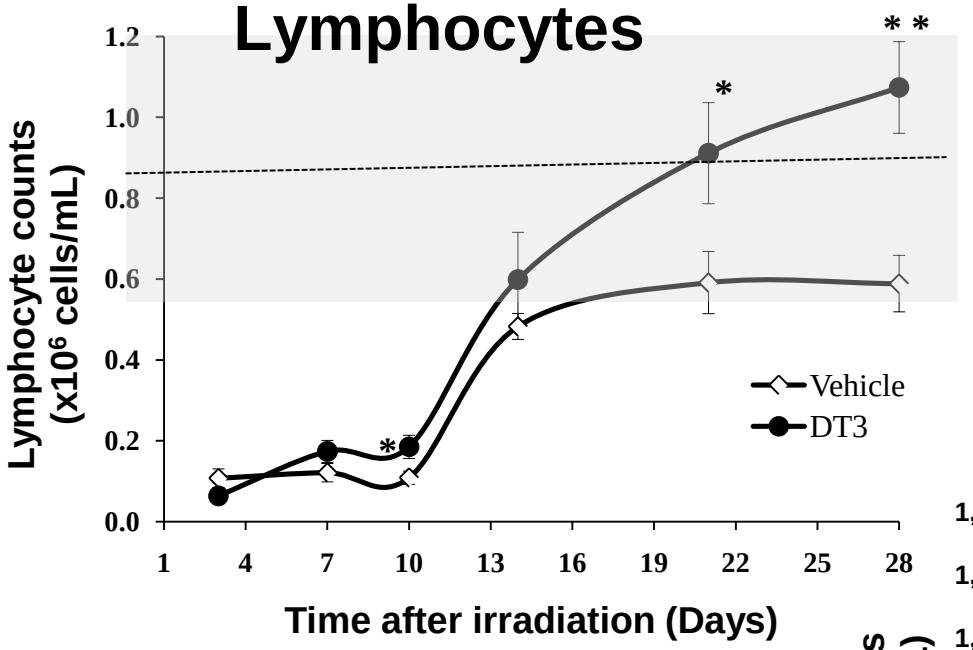
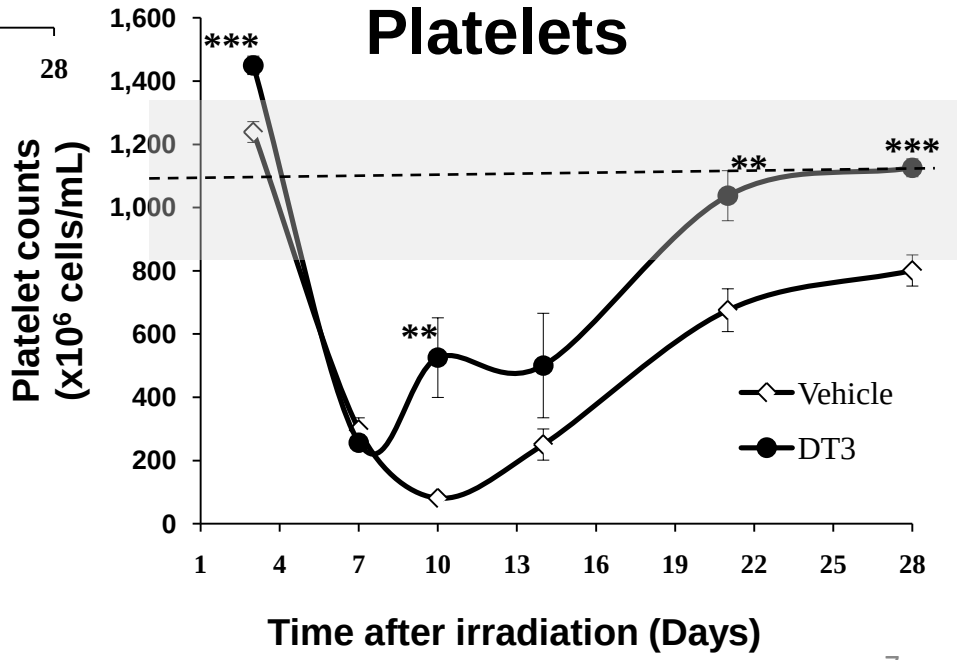
Hematology (Ctd)

DT3- 300 mg/kg, -24 h, sc , 7 Gy @ 0.6 Gy/min





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Preliminary studies

Oral formulations

Pharmacokinetics (SC and Oral)

Survival studies
with oral formulations

Oral formulations of DT3

(in collaboration with Yasoo health)

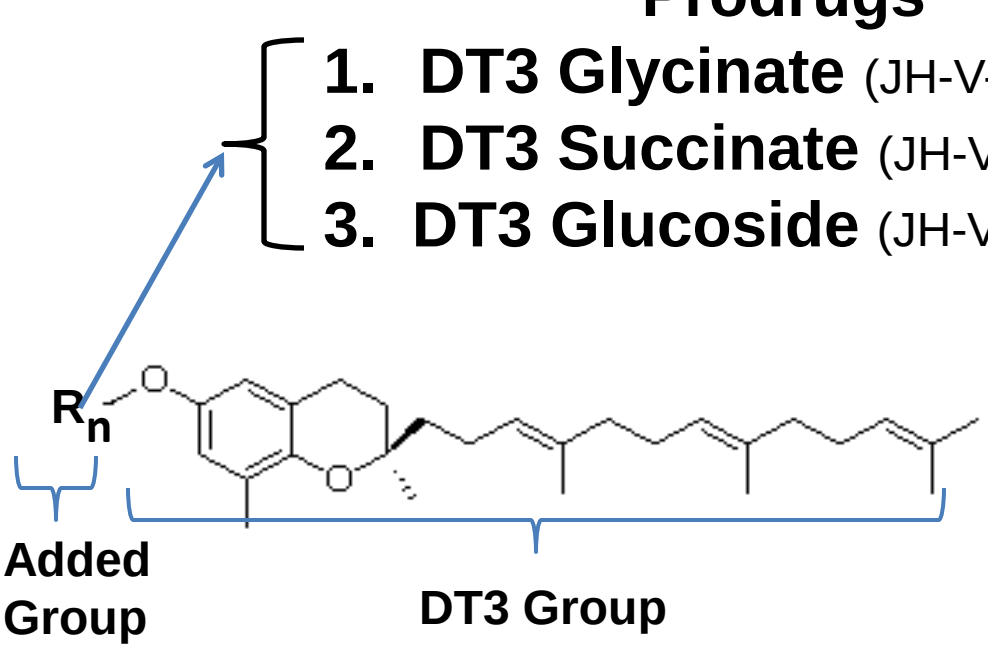
Emulsions

1. **DT3 in Emulsifier 1** (JH-V-107)
2. **DT3 in Emusifier 2** (JH-V-101.3)

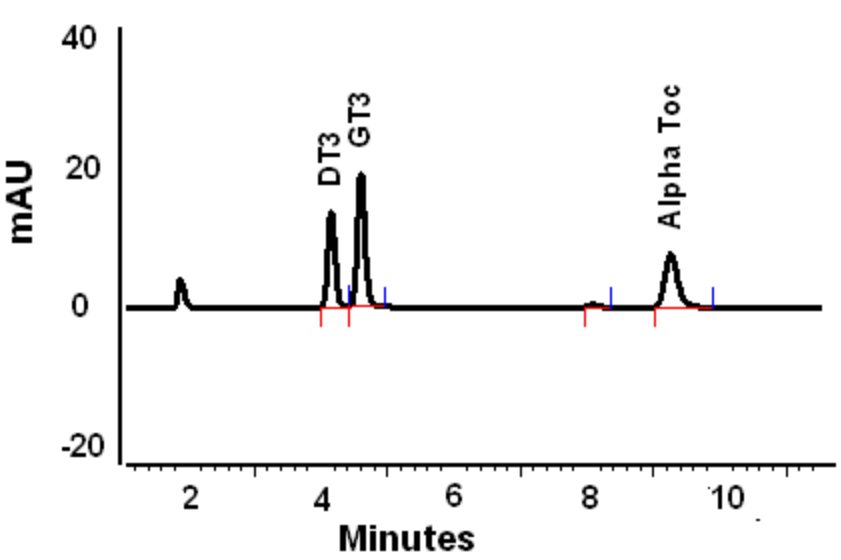
Tween 80, Brij combinations

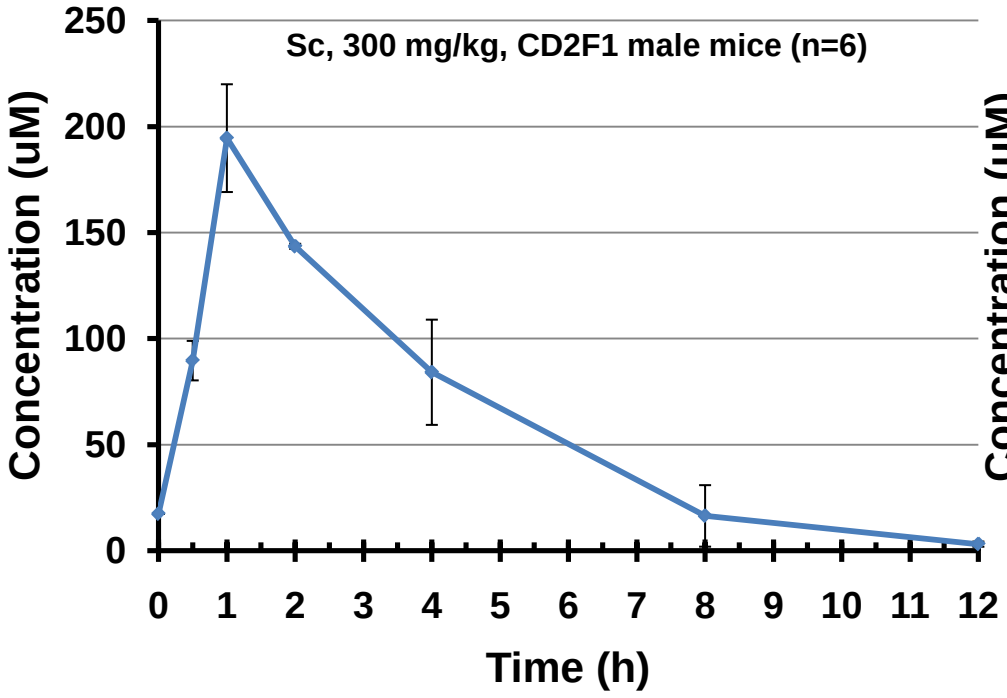
Prodrugs

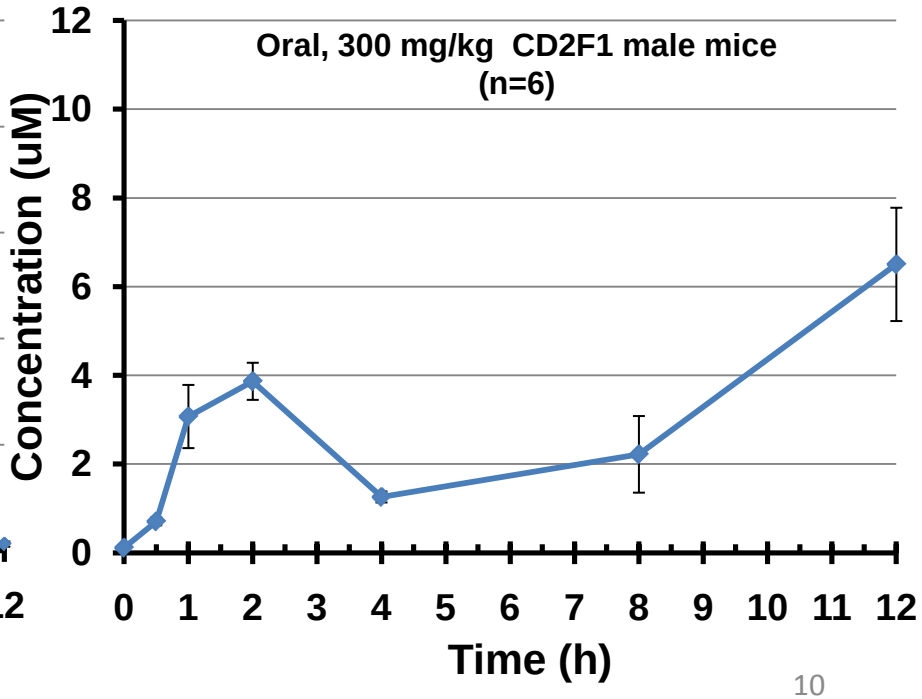
1. **DT3 Glycinate** (JH-V-91.2)
2. **DT3 Succinate** (JH-V-103.2)
3. **DT3 Glucoside** (JH-V-119)



HPLC analysis and Pharmacokinetics (Sc and oral) of DT3

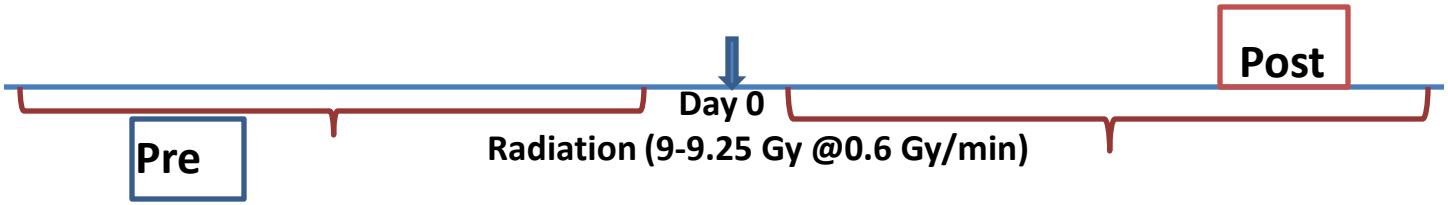






Survival: Oral formulations
(% 30 day)

Design: Pre- 4 daily gavage (last dose -24 h)
Post- 4 daily gavage (first dose 1 h postirradiation)



	DT3 dose (mg/kg)	DT3 in emulsifiers (EM) Vehicle	Pre	Post
EM1	75	25	25	56
EM2	100	25	25	25
		DT3 as Prodrugs		
Glycinate	225	6	6	19
Succinate	240	19	13	38
Glucoside	225	37*	38	44 (75)

* contains 10% ethanol

Introduction
(part 2- Xiao)

Promising candidates identified in a rodent system require further extensive mechanistic studies for FDA approval under the Animal Efficacy Rule.

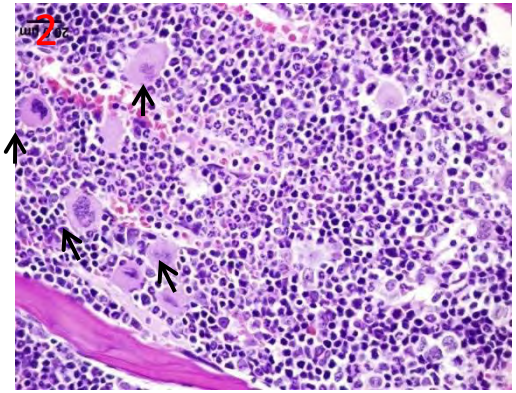
In the present study, we investigated the radioprotective mechanisms of DT3 on γ-irradiated CD2F1 mouse bone marrow and human hematopoietic progenitor CD34+ cells.

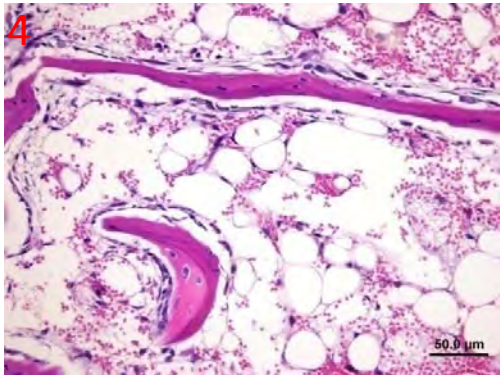
Mouse bone marrow (sternum) pathological changes

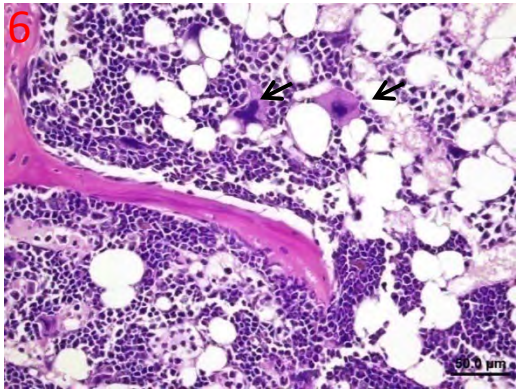
Control, 0 Gy

Vehicle, 8.75 Gy

DT3, 8.75 Gy

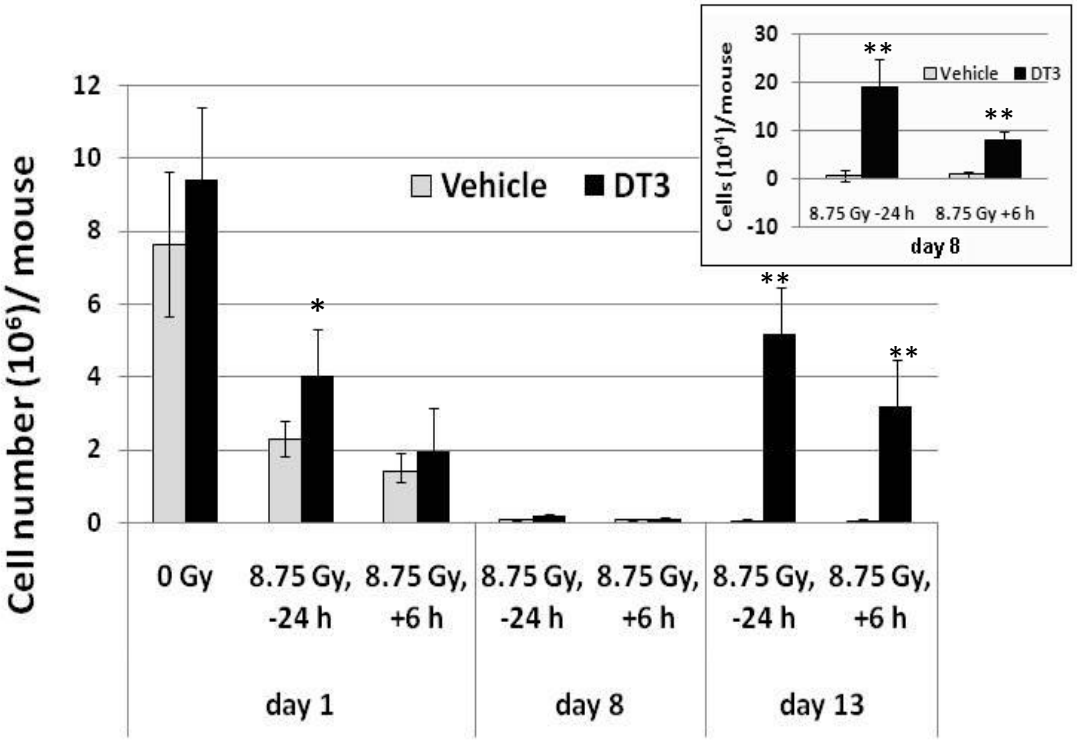


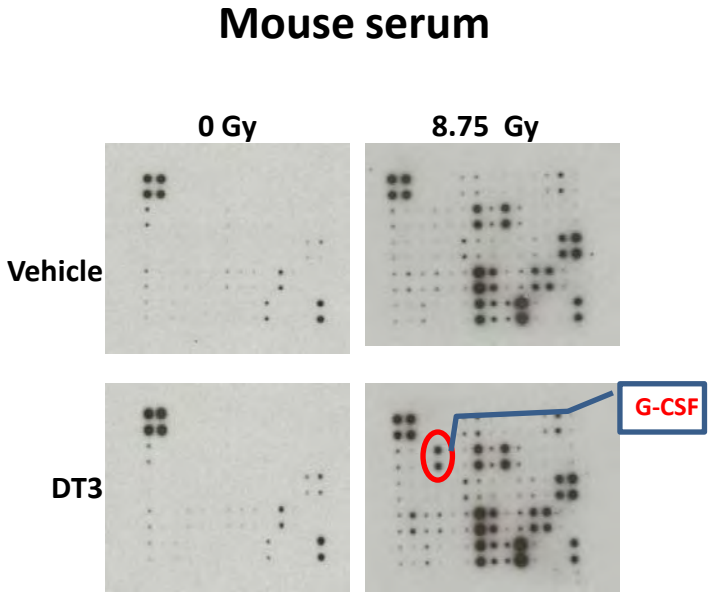




8 days post-irradiation

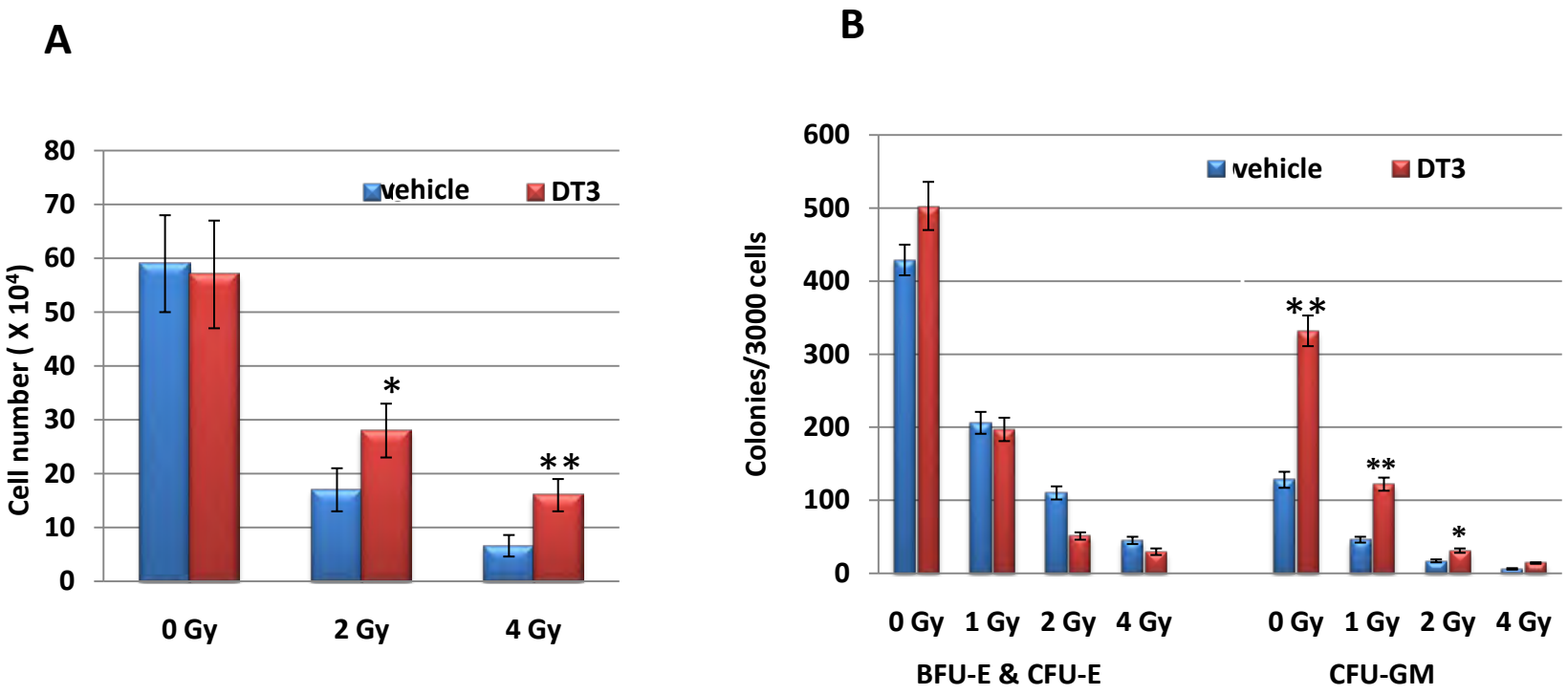
Effects of DT3 on recovery of mouse bone marrow myeloid cells from radiation damage (*in vivo*)





DT3 300mg/kg, SC. N = 6

DT3 protected human hematopoietic progenitor CD34+ cells (*in vitro*) from radiation damage

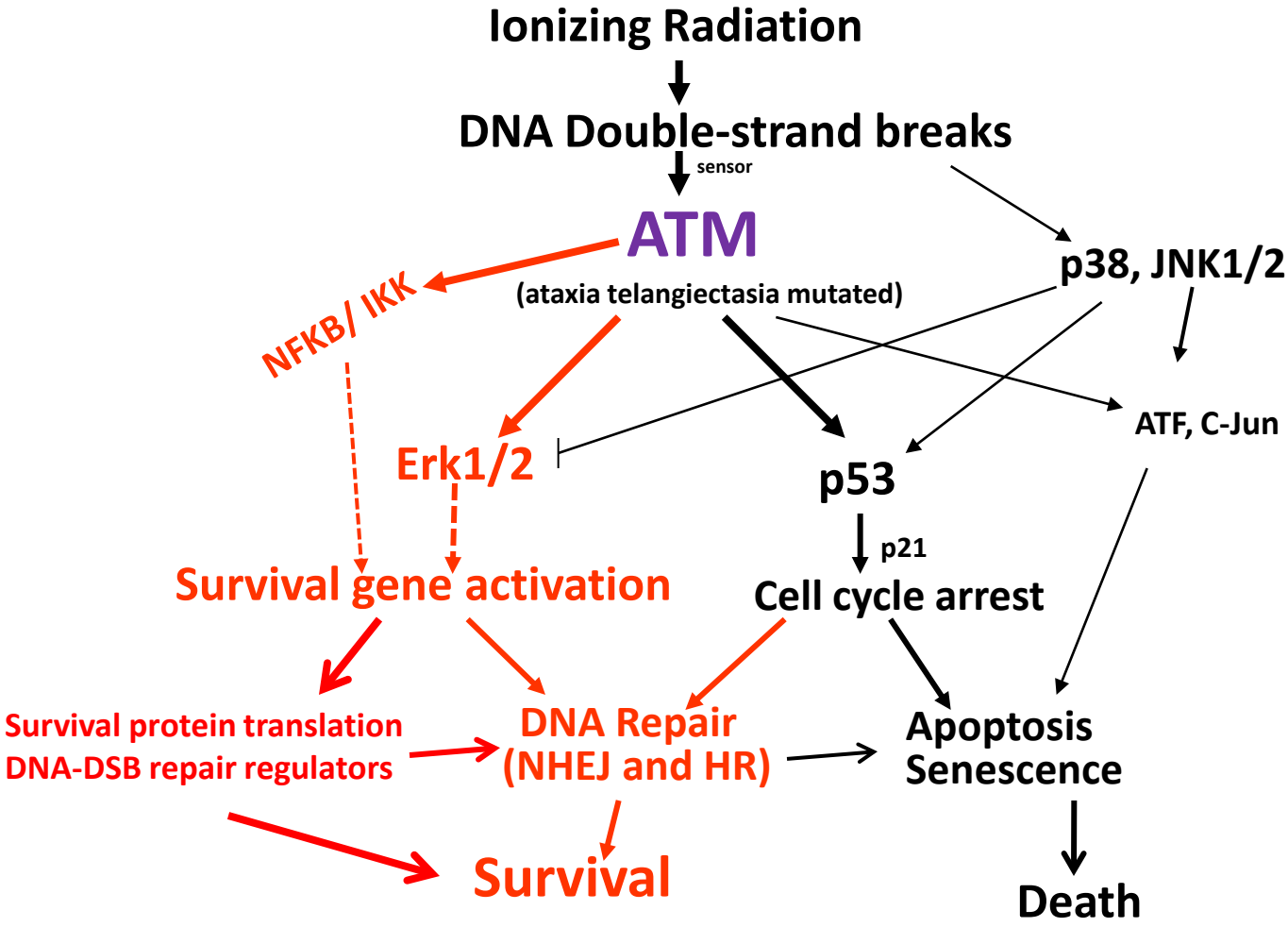


DT3 2 µM/mL, 24 h before radiation

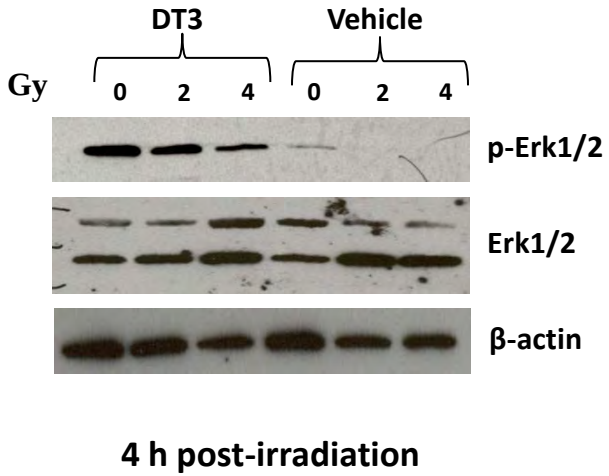
Survival of γ-irradiated mouse bone marrow and primary human hematopoietic CD34+ cells was significantly enhanced by Delta-tocotrienol (DT3).

Mechanisms?

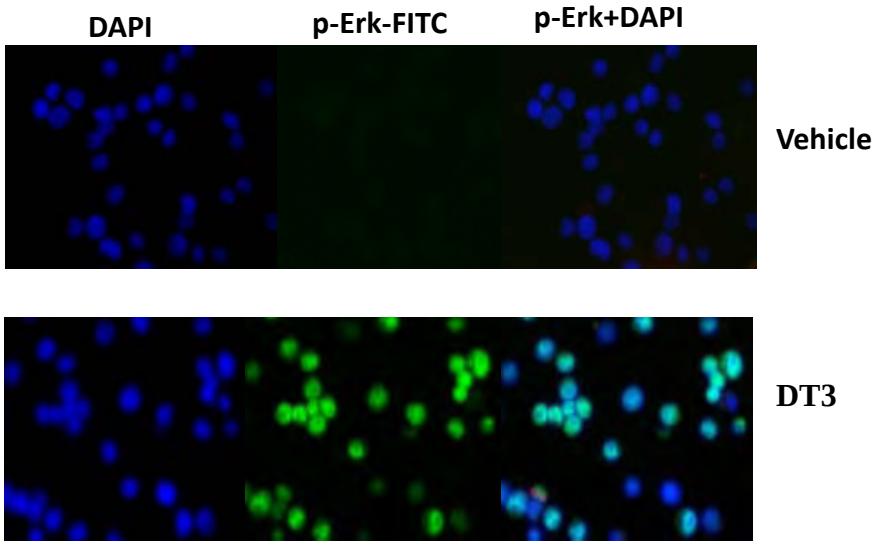
Radiation-induced activation of intracellular signal pathways



DT3 induced Erk1/2 phosphorylation in CD34+ cells

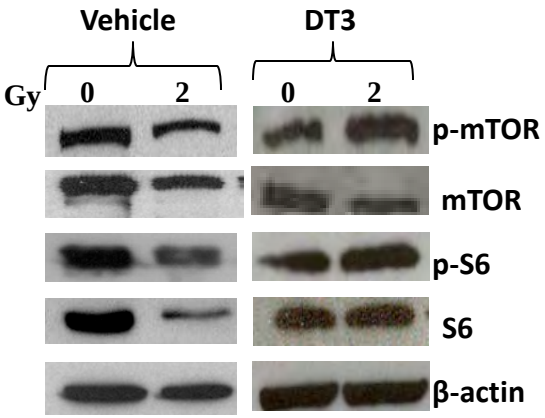


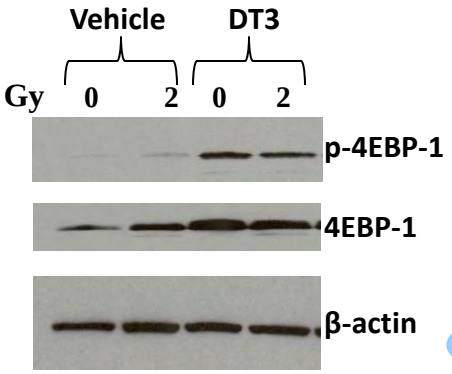
4 h post-irradiation

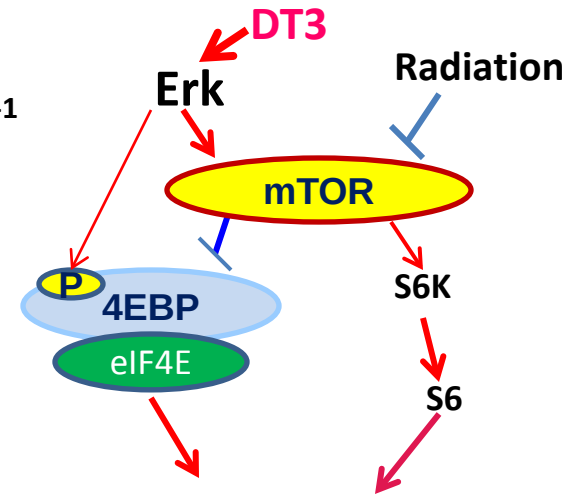


Normal human hematopoietic CD34+ cells have little or no Erk phosphorylation [Ricciardi et al. Leukemia. 2005;19:1543-1549**], and the phosphorylated Erk expression was very low or undetectable after γ-irradiation in CD34+ cells.**

DT3 reversed the radiation-inhibited mTOR and S6 protein activation and induced 4EBP-1 phosphorylation in CD34+ cells



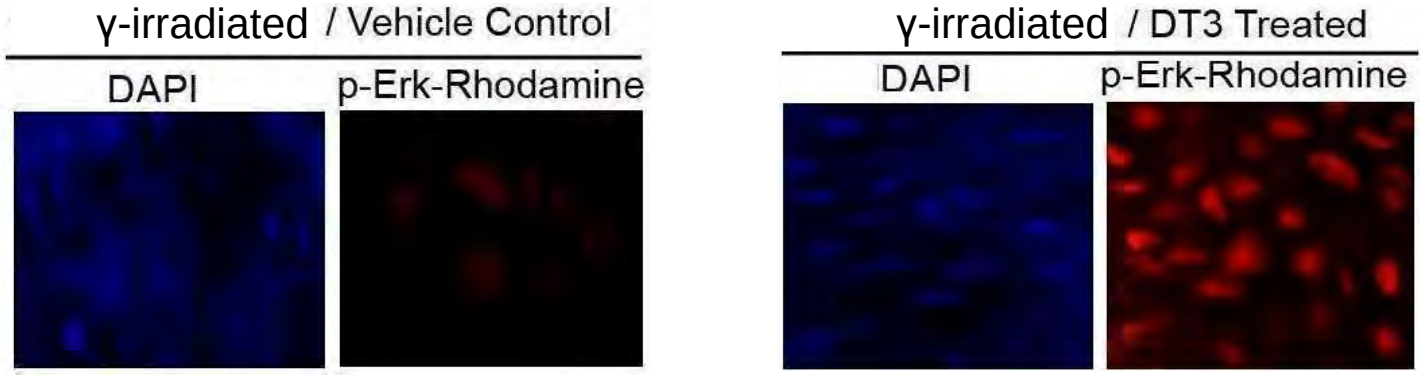


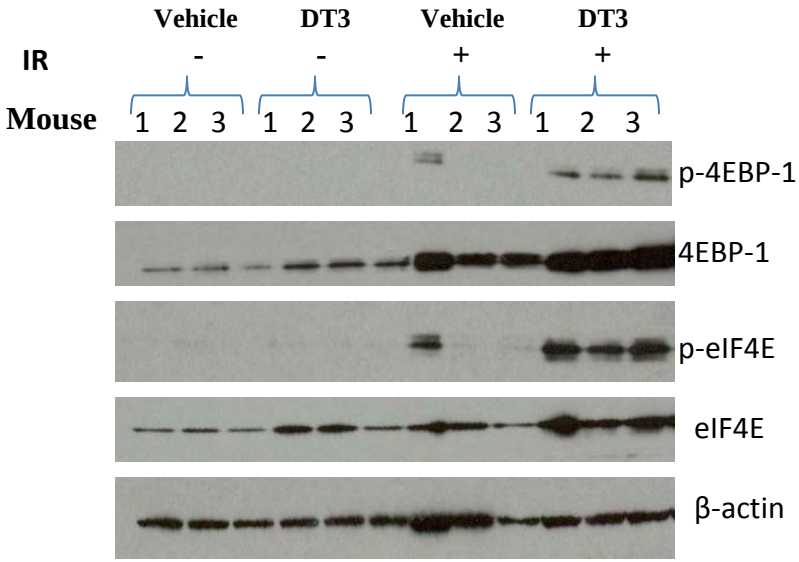


mTOR: Mammalian target of rapamycin
4EBP-1: Eukaryotic translation initiation factor 4E-binding protein 1
S6: Ribosomal protein

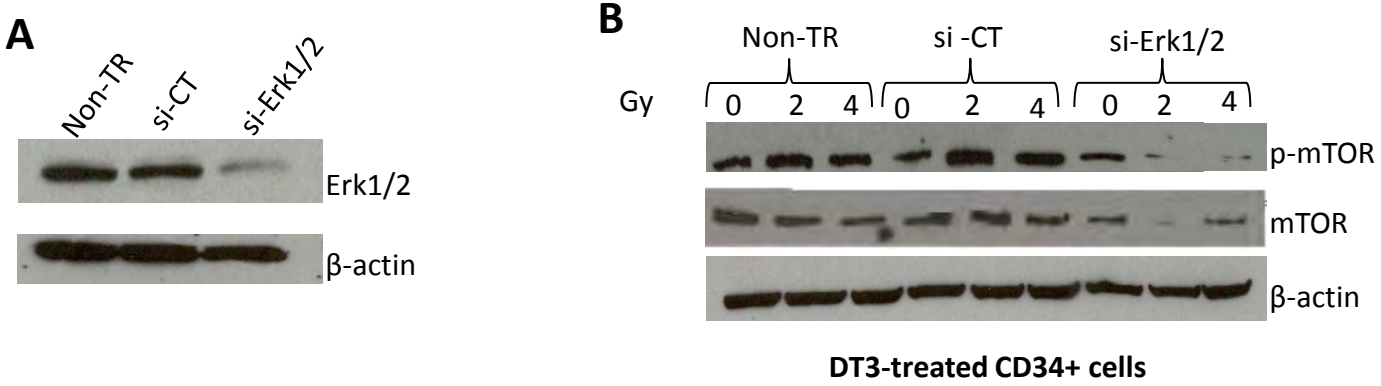
mTOR is a member of the phosphoinositid 3-OH kinase (PI3K)-related kinase family factors which involved in cell proliferation, cell cycle progression, DNA damage checkpoints and cell survival and growth.

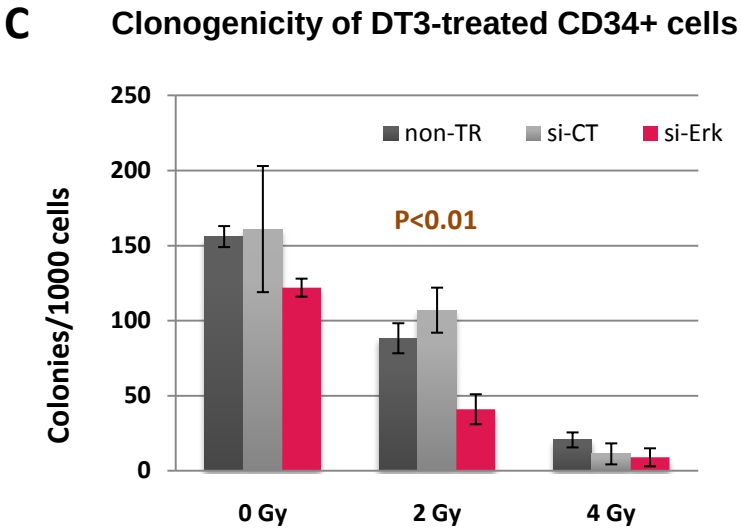
DT3 induced Erk phosphorylation in mouse bone marrow cells after irradiation (*in Vivo*)



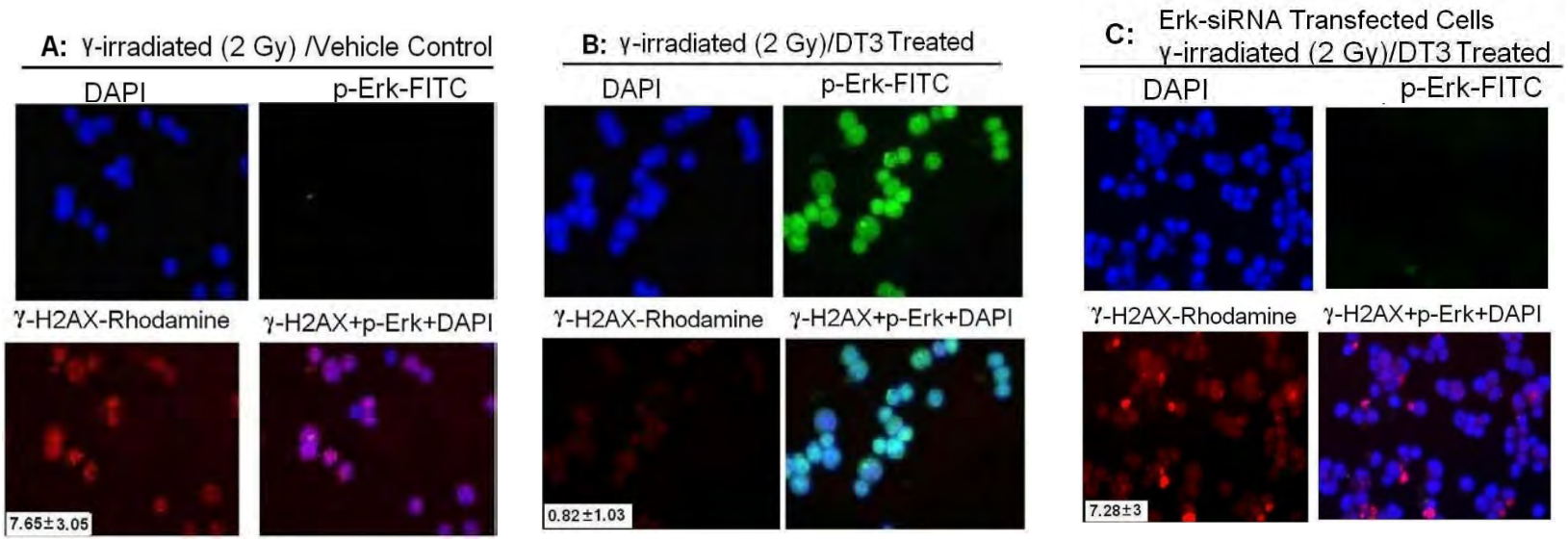


Erk gene knockdown suppressed m-TOR phosphorylation and clonogenicity in DT3-treated CD34+ cells after IR





DT3 induced Erk phosphorylation in γ-irradiated CD34+ cells and protected cells from irradiation-induced DNA-damage



Immunofluorescence staining using anti-γ-H2AX-Rhodamine (red) and anti-phospho-Erk-FITC (green) antibodies. DAPI (blue) defined the cell nucleus.

Summary and Conclusions

- Survival of γ-irradiated mouse bone marrow and primary human hematopoietic CD34+ cells was significantly enhanced by Delta-tocotrienol (DT3).

- DT3 dramatically induced Erk phosphorylation and decreased the DNA-damage marker γ-H2AX foci formation.

- DT3 reversed the radiation-inhibited mTOR and S6 protein activation, and induced 4EBP-1 phosphorylation.

- Knockdown of *Erk* gen*e* expression by siRNA abrogated DT3-induced mTOR phosphorylation, induced γ-H2AX foci formation, and inhibited clonogenicity in CD34+ cells.

In conclusion, our data suggest DT3 effectively protects mouse bone marrow and human CD34+ cells from radiation damage through the Erk/mTOR survival pathway (Hematological 2010; 95(12) 1996-2004).

Acknowledgments

AFRRI

Radiation Countermeasures Program

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